Appendix A:

Synthetic Control Design Parameters: Exemplary PCR Components and Reaction Parameters

1. Components (25µl reaction)

dH_2O	14.5µl
10X PCR Buffer	2.5µl
$MgCl_2$ (25mM)	2.5µl
dNTPs (2.0 mM)	2.5µl
Primers (10µM)	1.0µl
Amplitaq Gold 0.6U/µl)	1.0µl
Template (10µM mix)	1.0µl

2. Reaction Parameters

94°	10 minutes	
94°	45 seconds	5 Cycles
60°	45 seconds	
72°	45 seconds	
94°	45 seconds	25 Cycles
60°	45 seconds	
72°	45 seconds	
72°	5 minutes	
4°	∞	

Appendix B:

Synthetic Control Design Parameters: Exemplary Ligation Extension Components and Reaction Parameters

1. Components (20μl reaction)

CF ligation mixture	10μl
Bridge template (PCR amplicon / concentrated bridge oligo)	5.0μl
Reference nucleic acid mix (0.5μM)	5.0μl

2. Reaction Parameters

90°	5 seconds	32 Cycles
46.5°	45 seconds	
99°	10 minutes	
4°	∞	

Appendix C:

Synthetic Control Design Parameters: Exemplary Overlap Extension Components and Reaction Parameters

1. Components (25µl reaction)

dH_2O	16.5µl
10X PCR Buffer	2.5µl
$MgCl_2$ (25mM)	2.5µl
dNTPs (2.0 mM)	2.5µl
Oligonucleotides (10µM)	1.0µl
Amplitaq Gold 0.6U/µl)	1.0µl

2. Reaction Parameters

94°	10 minutes	
94°	45 seconds	5 Cycles
60°	45 seconds	
72°	45 seconds	
94°	45 seconds	25 Cycles
60°	45 seconds	
72°	45 seconds	
72°	5 minutes	
4°	∞	